9/745349

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Term: l1 and homologous recombina$5

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Search History

DATE: Tuesday, July 29, 2003 Printable Copy Create Case

Set Name side by side	Query	Hit Count	Set Name result set
DB=USPT,JPAB,EPAB,DWPI; PLUR=YES; OP=ADJ			
L3	l1 and homologous recombina$5	34	L3
L2	L1 and homolog$3	94	L2
L1	complementary near5 insert$1 near5 vector$1	123	L1

END OF SEARCH HISTORY

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L3: Entry 32 of 34 File: USPT Feb 2, 1999

DOCUMENT-IDENTIFIER: US 5866383 A
TITLE: In vitro ligation of foreign DNA into large eukaryotic viruses

Brief Summary Text (4):
Recombinant DNA technology has made it possible to express genes of one organism within another. The prior art shows that several virus groups, including the papovaviruses, papiluoma viruses, adenoviruses and retroviruses have been employed as eukaryotic molecular cloning and expression vectors. The relatively small sizes of these virus genomes have facilitated the in vitro construction of recombinant DNA molecules. However, these vectors generally exhibit a limited host range, provide severe limitations on the amounts of DNA that can be accommodated and lose their ability to infect subsequent cells upon the insertion of foreign DNA. Genetic engineering using larger viruses, i.e. those having genomes larger than about 50 kbp, such as poxviruses, herpesviruses or baculoviruses, is more difficult because of the large genome size. However, unlike viruses with smaller virus genomes, the larger virus genomes have a greater capacity for accommodating large foreign nucleic acid. sequences. Poxviruses are particularly useful because the viruses can infect a wide range of host cells. For poxviruses, such as vaccinia virus, past methods for generating recombinants have employed homologous recombination.

Brief Summary Text (5):
Prior to the present invention, homologous recombination in vivo was used to introduce foreign DNA into the genomes of large DNA viruses such as herpesviruses, poxviruses, and baculoviruses (reviewed by Miller, Bioessays 11: 91-5 (1989); Moss, Science 252: 1662-7 (1991) and Roizman, et al., Science 229: 1208-14 (1985)). Homologous recombination requires a number of genetic manipulations no longer required by the methods disclosed herein. As a first step for practicing homologous recombination, a vector is prepared separate from the viral genome. The vector is preferably a plasmid and in a poxvirus system, the plasmid is modified to contain a poxvirus promoter, sites for insertion of a foreign gene, and poxvirus DNA flanking sequences. A foreign gene is next inserted into the vector to form a chimeric gene and this construct is transfected into cells infected with a poxvirus having sequences complementary to the DNA flanking sequences. The progeny poxviruses are collected and tested for the presence of the foreign gene.

Brief Summary Text (6):
Direct in vitro ligation of DNA, into large eukaryotic viral genomes has not been used. It has heretofore been thought that such ligation techniques are not possible because of the difficulty of working with vector genomes of 50 to 200 kilobase pairs (kbp). With longer DNA molecules there is an increase in the number of times that a particular restriction endonuclease recognition site appears in the molecule and this creates significant problems for vectors created using methods other than homologous recombination. There has been a long felt belief in the scientific community that large genomes of this size are incapable of efficient direct ligation. For example, European Patent Application No. 0 443 335 to Bodemer indicates that the size of the vaccinia genome makes the construction of recombinant genomes by cleavage with restriction endonucleases and subsequent ligation with foreign DNA impossible. Accordingly, homologous recombination methods have been used to insert foreign DNA into viruses having greater than 50 kbp.

Brief Summary Text (7):
There are, however, significant problems associated with homologous recombination. For example, the overall efficiency of homologous recombination is low and the efficiency continues to decline further with increasing insert length. Moreover, the exact site of incorporation of a foreign DNA insert can only be predicted within a given region of the vector genome. The exact site of incorporation varies for each individual

recombination event within that given region. For this reason, considerable effort has gone into the development of selection and screening methods. Another potential drawback of homologous recombination protocols is that they generally require an intermediate cloning step followed by propagation of the DNA in bacteria where deletions or rearrangements may occur. Deletions and rearrangements are even more prevalent when the DNA has an unusual structure or is very large. These intermediate plasmid cloning steps make the production of cDNA expression libraries extremely labor intensive.

Brief Summary Text (8):
Recombinant viruses with large genomes, such as vaccinia virus, that have been generated by homologous recombination, have been shown to be useful as vaccines to generate protective immune response against the organisms from which the foreign DNA of the chimeric gene was derived. Some examples of such foreign genes include nucleic acid sequences encoding protein from hepatitis B virus, hepatitis A virus, hepatitis non-A, non-B virus, influenza virus, herpesvirus, cytomegalovirus, adenoviruses, parvoviruses, foot and mouth disease virus, poliovirus, measles virus, rabies virus, coronaviruses, coxsackieviruses and pathogenic bacteria, rickettsia, protozoa, and metazoa.

Brief Summary Text (11):
In one embodiment of the present invention, there is provided a method of inserting DNA into the genome of a DNA virus having a genome larger than about 50,000 base pairs. This method includes the steps of obtaining a viral DNA sequence having at least 50,000 base pairs, and cutting the viral DNA sequence at a single restriction endonuclease recognition site using a restriction endonuclease that recognizes the site. Thus, a first viral arm and a second viral arm are created. Insert DNA is obtained and ligated to the viral arms, with a first end of the insert ligated to the first arm and a second end of the insert ligated to the second arm. Preferably, the single restriction endonuclease recognition site is located in a region of the virus that is non-essential for replication of the virus. In certain embodiments, the viral DNA sequence initially includes several of the restriction endonuclease recognition sites in which DNA is to be inserted. If this is the case, the method also includes the step of modifying all but one of the restriction endonuclease recognition sites so that only the one restriction endonuclease recognition site can be cut by a restriction endonuclease recognizing the sites, thereby creating a unique restriction endonuclease recognition site within the viral DNA sequence. This modifying step can be methylation of all of the restriction endonuclease recognition sites but the one restriction endonuclease recognition site into which DNA is to be inserted. The modifying step can also represent an addition, deletion or base change within all of the restriction endonuclease recognition sites but the one restriction endonuclease recognition site. In some embodiments, the modifying step is accomplished by homologous recombination with a DNA sequence containing the addition, deletion or base change. In embodiments where the viral DNA sequence has a unique restriction endonuclease recognition site, the cutting step can be accomplished by complete digestion of the DNA sequence with a restriction endonuclease that recognizes the unique restriction endonuclease recognition site. In an especially preferred embodiment, the unique restriction endonuclease recognition site is located within a marker gene associated with a phenotype, and the method includes the step of identifying viral arms containing insert DNA by identifying a change in the phenotype, such as the development of color. The viral DNA sequence can be a sequence from any of a number of viruses, including a Poxvirus, such as vaccinia virus. In a preferred embodiment, the ligated DNA is transfected into a susceptible host cell. Preferably, the susceptible host cell is infected with a helper virus that enables replication of the transfected DNA. The helper virus can be conditionally lethal to aid in the selection process. In a preferred embodiment of the method, the helper virus does not allow replication of viral DNA in the presence of a functional thymidine kinase gene. Preferably, the transfected DNA can replicate essentially without recombination with the genome of the helper virus. The helper virus can be recombination deficient under at least one condition, such as at a particular temperature, e.g. above 31.degree. C.

Detailed Description Text (3):
To circumvent the limitations inherent in the generation of chimeric viral genomes by a homologous recombination protocol, a unique restriction endonuclease site was incorporated into the nearly 200,000 bp vaccinia virus genome. Cleavage at this unique

restriction endonuclease site produces two viral DNA arms that could be ligated with foreign DNA inserts that preferably operably encoded protein to produce a chimeric viral genome.

Detailed Description Text (8):
The term "homologous recombination" is a term of art denoting a method or procedure for incorporating a sequence of nucleic acid into a target sequence. For a description of homologous recombination see Moss, B. Science 252: 1662-1667 (1991) which is hereby incorporated by reference.

Detailed Description Text (16):
In a preferred embodiment of this invention, the invention comprises obtaining a viral DNA sequence of at least 50 kbp and cutting the DNA sequence at a single restriction endonuclease recognition site to create two viral arms. These arms are ligated to insert DNA and the resulting construct is used to generate recombinant virus and to express a foreign nucleic acid sequence in a cell. We have demonstrated that very large inserts, e.g. at least 26,000 base pairs, can be accomodated using the methods of the present invention to produce recombinants at high efficiency. This is unlike prior art homologous recombination techniques, in which efficiency of recombination decreased drastically with increasing insert size. It is believed that the efficiency of incorporation of inserts greater than about 20,000 base pairs using prior art homolgous recombination techniques is extremely low. Accordingly, Applicants believe that detection and isolation of recombinant viruses having inserts larger than about 20,000 base pairs using homologous recombination techniques would be so difficult as to be effectively prohibited.

Detailed Description Text (21):
In Example 1, the NotI restriction endonuclease site was removed from the vaccinia genome and replaced with a 4 bp insert using homologous recombination. Since the NotI site was located in a portion of the genome that had not been mapped for open reading frames, it was necessary to first determine if the restriction site was located in an essential region of the genome. Essential portions of the genome are those that include open reading frames or regulatory regions of the genome and are required for virus replication and infection. There are several non-essential portions of the vaccinia genome that have been disclosed in the art. Restriction endonucleases located in these non-essential regions of the vaccinia genome can be modified without disrupting the infectivity of the progeny virus or the ability of the progeny virus to replicate. Examples of such non-essential regions include the thymidine kinase gene and a region of at least 9,000 base-pairs (bp) that is proximal to the left inverted terminal repetition as well as intergenic regions located throughout the genome.

Detailed Description Text (23):
As another preferred method for determining whether a restriction endonuclease site is located in a nonessential portion of a large viral genome, an insertion cassette, such as a .beta.-galactosidase cassette, can be incorporated into the restriction site, preferably by homologous recombination. As a preferred example, the incorporated cassette includes a region of nucleic acid operably coding for a foreign protein that facilitates the selection of viral genomes. This foreign protein may be a marker protein such as a thymidine kinase, Escherichia coli LacZ, E. coli xanthine-guanine phosphoribosyl-transferase (gpt), luciferase or the like.

Detailed Description Text (24):
In a particularly preferred example, a DNA sequence was inserted by homologous recombination into the NotI restriction endonuclease site located in the HindIII F fragment. This DNA sequence consisted of the vaccinia virus P11 promoter derived from the gene encoding the 11K structural protein (Wittek, et al., J. Virol 49: 371-378 (1984), hereby incorporated by reference) regulating the Escherichia coli lacZ gene. FIG. 1 is a schematic outlining a preferred strategy for the production of two vaccinia expression vectors vNotI/lacZ/tk and vNotI/tk suitable for in vitro ligation to a foreign nucleic acid insert following NotI digestion. Step 1 illustrates the viral genome before and after homologous recombination using the plasmid pF-lacZ containing the P11-lacZ gene cassette derived by digestion of pSC20 (Buller et al., see Example 1) with Bgl II in the NotI site of the HindIII F fragment. .beta.-galactosidase activity was used as a marker for recombinant virus selection and Southern analysis of DNA obtained form the virus plaques using lacZ gene fragments as

probes confirmed the identity of the positive clones. A clone, vMM1, was selected that had the lacZ gene inserted into the NotI site as illustrated in FIG. 1. The ability to isolate a recombinant vaccinia virus containing an insert at the NotI site implied that the DNA immediately surrounding this restriction site was not required for replication. This region, HindIII F, was then sequenced and it was confirmed that the NotI site was in a region not required for protein expression (Goebel et al., see Example 1). Such methods can similarly be used by one of skill in the art, to determine whether or not a particular region of nucleic acid from any large virus genome (>50 kbp) is essential to progeny virus production.

Detailed Description Text (26):
Similar results can be obtained using any number of methods known in the art for deleting, substituting or adding base pairs within a particular restriction endonuclease site. Thus, restriction fragments from a large viral genome that contain a restriction site to be modified can be isolated after fragment separation on an agarose gel. The fragment can be incorporated into a working plasmid such as pBluescript (Stratagene, LaJolla, Calif.) or the like and the restriction site can be modified by oligonucleotide selection employing the polymerase chain reaction or by any number of site-directed mutagenesis strategies known to those in the art. The modified fragment is then incorporated into a suitable vector and introduced into the large viral genome by homologous recombination.

Detailed Description Text (33):
This step was accomplished using homologous recombination involving the transfection of plasmid pSC41, an intermediate in the construction of pUV1 (Falkner et al., see Example 2), containing the tk gene interrupted by a cassette consisting of a P11 promoter-lacZ gene with an in-frame NotI site at the fifteenth codon. Because of the anticipated tk.sup.- phenotype, 5-bromodeoxyuridine (BUdR) was used in conjunction with tk.sup.- cells for selection of recombinant virus plaques (Earl et al., Current Protocols in Molecular Biology ("Current Protocols"), 16.15.1-16.18.10, 1990, hereby incorporated by reference, and Example 2). In additional X-Gal staining confirmed that the .beta.-galactosidase fusion protein was active. One such NotI+, lacZ+, tk.sup.- recombinant virus, named vNotI/lacZ/tk, was purified and amplified.

Detailed Description Text (36):
A second exemplary vector suitable for direct in vitro ligation was constructed based on thymidine kinase selection. The second vector, vNotI/tk, permits direct cloning of NotI fragments into vaccinia virus and selection for tk.sup.- cells. As disclosed in Example 1 and illustrated in FIG. 3B, a unique in frame NotI site was engineered using homologous recombination to introduce the tk gene containing a NotI site immediately after the tk translation initiation codon. Using methods identical to those described for the production of vNotI/lacZ/tk, the resulting plasmid was transfected into cells that had been infected with vNotI/lacZ/tk. In contrast to the latter virus, which is lacZ.sup.+ and tk.sup.-, the new recombinants were expected to be lacZ.sup.- and tk.sup.+. tk.sup.- cells were grown in HAT medium (Weir et al., see Example 2) to specifically select tk.sup.+ virus plaques, see Example 4. DNA from a tk.sup.+ plaque isolate was analyzed by restriction endonuclease analysis and the resulting electrophoretic pattern of the digest is provided in FIG. 2B and confirmed by DNA sequencing (FIG. 3B).

Detailed Description Text (38):
It is additionally contemplated that a second unique restriction endonuclease site can also be incorporated into the viral vector preferably downstream from the first unique restriction site and still more preferably also within the marker protein. Incorporation of the second restriction endonuclease site into the vector provides a method for directed insert incorporation using inserts with ends complementary to the restriction endonuclease. The presence of two restriction endonuclease sites is particularly useful for the incorporation of an insert in a defined orientation. Still further, it is also contemplated that the viral vector may incorporate multiple cloning sites within a suitable marker protein to facilitate the insertion of a variety of restriction endonuclease fragments.

Detailed Description Text (41):
The plasmids were digested with NotI, ligated separately to the vaccinia virus DNA arms and precipitated with calcium phosphate. The precipitated DNA was applied to

monolayers of CV-1 cells that were infected with ts42, a conditionally lethal temperature sensitive vaccinia virus mutant, that is defective in DNA replication (Condit et al., Sridhar et al., and Traktman et al., see Example 7) and homologous recombination at 40.degree. C.

Detailed Description Text (42):
Helper viruses are those viruses that together with the transfected nucleic acid provide appropriate proteins and factors necessary for replication and assembly of progeny virus. Conditionally lethal helper virus was previously used to facilitate the isolation of viruses with genomes formed by homologous recombination between co-transfected plasmid and wild type DNA (Fathi, et al., Virol 155: 97-105 (1986) and Kieny, et al., Nature 312: 163-166 (1984), both hereby incorporated by reference). Incubation of ts42 infected cells transfected with the constructs of this invention and incubation at 40.degree. C. prevents recombination between the helper virus and the recombinant viral vector. Following infection, the cells were incubated until progeny virus was produced and the cells were harvested. The virus titer was determined by plaque assay on cell lines at 37.degree. C.

Detailed Description Text (47):
This invention, advantageously provides a method for easily inserting large DNA fragments into large viral genomes. Insertion of large DNA fragments is difficult by homologous recombination. However, using the methods of this invention, the efficiency of incorporating a 26 kbp insert was similar to the efficiency of incorporating a 4.3 kbp insert using the methods of this invention. This level of insert efficiency is not available by homologous recombination. The size of the insert incorporated into large viral genomes, such as the Poxviruses and the Herpesvirus family may only be limited by the physical constraints of virus assembly on the genome size. A large drop in efficiency was noted when a DNA fragment approaching 50 kbp was used in the vaccinia system. If the packaging capacity is the limiting factor to insert size, use of vaccinia virus deletion mutants (Perkus, et al., Virology 180: 406-410 (1991), hereby incorporated by reference) should allow the limit to be increased another 25 to 50 kbp.

Detailed Description Text (53):
Step 1 illustrates the viral genome before and after homologous recombination using the plasmid pF-lacZ containing the P11-lacZ gene cassette derived by digestion of pSC20 (Buller, et al., J. Virol 62: 866-874, 1988 hereby incorporated by reference) with Bgl II in the NotI site of the HindIII F fragment. The progeny were analyzed by plaque assay (see Current Protocols. p. 16.16.5). Plaques that turned a blue color with X-Gal were isolated and expanded (see Current Protocols. pp.16.17.12-16.17.13).

Detailed Description Text (55):
Since the NotI site was non-essential, it was eliminated from vMM1 by homologous recombination with a plasmid containing the HindIII F fragment that had been cut with NotI and the recessed ends filled in with the Klenow fragment of DNA polymerase prior to re-ligation (see Maniatis, supra) (FIG. 1, step 2). Successful recombination resulted in a replacement of the P11 promoter-lacZ cassette with a 4 bp insertion that destroys the NotI site (for homologous recombination methods see Current Protocols pp. 16.17.1-16.17.6) These recombinants could no longer express .beta.-galactosidase, therefore, colorless plaques were picked from monolayers that also contained parental-type plaques that stained blue with X-Gal. Restriction enzyme analysis and DNA blotting of the viral DNA of vNot-confirmed the loss of the lacZ gene and the NotI site. (see Maniatis et al., supra and Current Protocols. pp. 16.18.3-16.18.4)

Detailed Description Text (63):
Transfection Procedure and Homologous Recombination

Detailed Description Text (64):
Plasmids containing genes or nucleic acid to be inserted into the target vaccinia genome were flanked by DNA from sites having homology to the regions of DNA flanking the target site for recombination on the vaccinia genome. The insert was incorporated into the vaccinia virus genome by homologous recombination. Typically, confluent monolayers of CV-1, BSC-1, TK 143 (all available from American Type Culture Collection (ATCC), Rockville Md.), or other cells in bottles with a 25 cm.sup.2 bottom surface area were infected with 0.01 to 0.05 plaque forming units (pfu) per cell of vaccinia

virus. Approximately 5 .mu.g of plasmid DNA with or without carrier DNA (preferably appropriate viral DNA) was mixed in 1 ml of 0.1% dextrose, 0.14M NaCl, 5 mM KCl, 1 mM Na.sub.2 HPO.sub.4, 20 mM Herpes, (pH 7.05) and precipitated by addition of CaCl.sub.2 to a final concentration of 125 mM. The mixture was agitated gently and allowed to remain at room temperature for about 30 min. Two hr after infection, 0.8 ml of the fine suspension was added to an infected monolayer overlayed with 5 ml of media. After 4 hr., cell media was replaced with 5 ml of Eagle or other tissue culture medium containing 8% fetal bovine serum was added to each bottle and the incubation was continued at 37.degree. C. for 48 more hr. At this time, the infected cells were scraped off the bottle, centrifuged, resuspended in tissue culture medium and lysed by three cycles of freeze thaw to liberate virus.

Detailed Description Text (78):
The precipitated DNA was applied to monolayers of CV-1 cells (ATCC) that had been infected 2 h earlier at a multiplicity of 0.05 with ts42, a conditionally lethal temperature sensitive vaccinia virus mutant that is defective in DNA replication (Condit, et al., Virology 128: 429-443 (1983); Sridhar, et al., Virology 128: 444-457 (1983) and Traktman, et al., J. Virol 63: 841-846 (1989) all hereby incorporated by reference) and homologous recombination (Merchlinksky, J. Virol 63: 2030-2035 (1989), hereby incorporated by reference) at 40.degree. C. After incubation at 40.degree. C. for 2 days, the cells were harvested and the virus titer was determined by plaque assay on cell lines at 37.degree. C.

Detailed Description Text (86):
The techniques for developing Herpesvirus (HSV) vectors follow the techniques outlined generally above. Many genes of HSV have been mapped to the viral genome. For a general review of the genome map with references see Roizman et al., "Herpes Simplex Viruses and Their Replication" in Fundamental Virology, Fields et al., eds. 1990, Raven Press, hereby incorporated by reference. Protocols are available for the site-specific insertion and deletion of nucleic acid inserts into the HSV genome by homologous recombination (Post et al., Cell 25: 227-232, 1981, and Roizman et al., Science 229: 1208-1214 (1985), both hereby incorporated by reference). In addition, a number of non-essential regions of the HSV genome have been identified. For a review of these regions see Roizman et al. in Fundamental Virology, supra. p. 860. Therefore, using site-directed mutagenesis, a technique well known in the art and available in kit form from manufacturers such as BioRad (Richmond, Calif.) or the like, a unique NotI restriction endonuclease site is inserted into the Herpesvirus thymidine kinase gene cloned into a plasmid suitable for homologous recombination with the HSV genome (see Poffenberg et al., Proc. Natl. Acad. Sci. USA 80: 2690-2694 (1983), hereby incorporated by reference). Other NotI sites located within the HSV genome are modified in non-essential portions of the genome by homologous recombination with plasmids containing the NotI site deleted by methods known in the art such as by cleaving the site, filling in with Klenow, and religating, as described in Example 1 and 2. For essential regions of the genome, the NotI site is modified by site-directed mutagenesis of plasmid fragments corresponding to sequences within essential regions of the genome followed by homologous recombination. Care is taken to ensure that the open reading frames of the essential portion of the genome are not interrupted by the deletion of the NotI sites.

CLAIMS:

19. The method of claim 16, wherein said adding, deleting, or changing a base comprises homologous recombination with a DNA sequence containing an added, deleted, or changed base.

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=> s complementary(10a)insert#(10a)vector#
L1 43 COMPLEMENTARY(10A) INSERT#(10A) VECTOR#

=> dup rem l1
PROCESSING COMPLETED FOR L1
L2 29 DUP REM L1 (14 DUPLICATES REMOVED)

=> s l2 and homologous recombina#####
L3 0 L2 AND HOMOLOGOUS RECOMBINA#####

=> s l3 and homologous
L4 0 L3 AND HOMOLOGOUS

=> d l2 1-29 bib ab kwic

L2 ANSWER 1 OF 29 CAPLUS COPYRIGHT 2003 ACS on STN
AN 2002:510425 CAPLUS
DN 138:33937
TI Microsatellite markers for the Paddlefish (Polyodon spathula)
AU Heist, Edward J.; Nicholson, Erica H.; Sipiorski, Justin T.; Keeney, Devon B.
CS Fisheries and Illinois Aquaculture Center, Southern Illinois University Carbondale, Carbondale, IL, 62901-6511, USA
SO Conservation Genetics (2002), 3(2), 205-207
CODEN: CGOEAC; ISSN: 1566-0621
PB Kluwer Academic Publishers
DT Journal
LA English
AB A suite of polymorphic genetic markers (microsatellites) has been developed for use in the efforts to conserve the American paddlefish. Tissue samples were collected from 28 individual American paddlefish. Polymorphic microsatellite loci was amplified in nine genomic DNAs from each of shovelnose sturgeon (Scaphirhynchus platorynchus) and green sturgeon (Acipenser medirostris) at an annealing temp. of 56.degree.. Twenty six of 1536 colonies strongly hybridized to the probe and were sequenced. Several loci were rejected because they had either no apparent repeat, short repeats, not enough sequence between the **vector** and the **insert**, or self-**complementary** flanking sequence that prevented the design of PCR primers. Primers were designed for fourteen loci, eight of which reliably amplified polymorphic microsatellite loci.
RE.CNT 7 THERE ARE 7 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT
AB A suite of polymorphic genetic markers (microsatellites) has been developed for use in the efforts to conserve the American paddlefish. Tissue samples were collected from 28 individual American paddlefish. Polymorphic microsatellite loci was amplified in nine genomic DNAs from each of shovelnose sturgeon (Scaphirhynchus platorynchus) and green sturgeon (Acipenser medirostris) at an annealing temp. of 56.degree.. Twenty six of 1536 colonies strongly hybridized to the probe and were sequenced. Several loci were rejected because they had either no apparent repeat, short repeats, not enough sequence between the **vector** and the **insert**, or self-**complementary** flanking sequence that prevented the design of PCR primers. Primers were designed

for fourteen loci, eight of which reliably amplified polymorphic microsatellite loci.

L2 ANSWER 2 OF 29 CAPLUS COPYRIGHT 2003 ACS on STN
AN 2000:191258 CAPLUS
DN 132:232707
TI Isolation of primer extension products with modular oligonucleotides in nucleic acid sequencing
IN Lundeberg, Joakim; Uhlen, Mathias
PA Dynal A/S, Norway; Jones, Elizabeth Louise
SO PCT Int. Appl., 74 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 2

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2000015842	A1	20000323	WO 1999-GB3056	19990915
	W: AE, AL, AM, AT, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, CZ, DE, DE, DK, DK, DM, EE, EE, ES, FI, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
	RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
	US 6482592	B1	20021119	US 1998-153242	19980915
	CA 2343072	AA	20000323	CA 1999-2343072	19990915
	AU 9960992	A1	20000403	AU 1999-60992	19990915
	EP 1114185	A1	20010711	EP 1999-947604	19990915
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, MC, IE, SI, LT, LV, FI, RO				
	JP 2002525076	T2	20020813	JP 2000-570369	19990915
PRAI	US 1998-153242	A	19980915		
	GB 1998-20185	A	19980916		
	WO 1997-GB2629	A2	19970926		
	WO 1999-GB3056	W	19990915		

AB The invention provides a method of isolating primer extension products, particularly sequencing reaction products, in which the products contain sequences corresponding or **complementary** to (i) a primer binding region, (ii) an **insert**, and (iii) **vector**-derived sequence(s), and a modular oligonucleotide of at least two parts which is complementary to the vector-derived sequence(s) and binds to adjacent stretches of the primer extension products is used for isolation, the modular oligonucleotides themselves and their use in methods of the invention. Two different approaches are taken to the design of modular oligonucleotides. Firstly, dedicated or specific modular oligonucleotides are designed which are specifically appropriate for use in large scale projects in which the same vector and cloning site of the insert are used extensively. In this case, the modular oligonucleotides anneal in parts of the multiple cloning site of the vector which are kept intact after cloning of the insert. The second approach to the design of suitable modular oligonucleotides to capture primer extension products is the prodn. of generic modular oligonucleotides which are able to bind to and capture primer extension products generated from any insert cloned into the multiple cloning site of a given vector. Thus, even the most extreme positioned restriction sites may be used for cloning.

RE.CNT 5 THERE ARE 5 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

AB The invention provides a method of isolating primer extension products, particularly sequencing reaction products, in which the products contain sequences corresponding or **complementary** to (i) a primer binding region, (ii) an **insert**, and (iii) **vector**-derived

sequence(s), and a modular oligonucleotide of at least two parts which is complementary to the vector-derived sequence(s) and binds to adjacent stretches of the primer extension products is used for isolation, the modular oligonucleotides themselves and their use in methods of the invention. Two different approaches are taken to the design of modular oligonucleotides. Firstly, dedicated or specific modular oligonucleotides are designed which are specifically appropriate for use in large scale projects in which the same vector and cloning site of the insert are used extensively. In this case, the modular oligonucleotides anneal in parts of the multiple cloning site of the vector which are kept intact after cloning of the insert. The second approach to the design of suitable modular oligonucleotides to capture primer extension products is the prodn. of generic modular oligonucleotides which are able to bind to and capture primer extension products generated from any insert cloned into the multiple cloning site of a given vector. Thus, even the most extreme positioned restriction sites may be used for cloning.

L2 ANSWER 3 OF 29 MEDLINE on STN
AN 1999412573 MEDLINE
DN 99412573 PubMed ID: 10481038
TI Enzyme-free cloning: a rapid method to clone PCR products independent of vector restriction enzyme sites.
AU Tillett D; Neilan B A
CS School of Microbiology and Immunology, The University of New South Wales, Sydney 2052, Australia.
SO NUCLEIC ACIDS RESEARCH, (1999 Oct 1) 27 (19) e26.
Journal code: 0411011. ISSN: 1362-4962.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199909
ED Entered STN: 19991005
Last Updated on STN: 20010521
Entered Medline: 19990917
AB We describe a simple method for the cloning of PCR products without the need for post-amplification enzymatic treatment. Tailed PCR primer sets are used to create **complementary** staggered overhangs on both **insert** and **vector** by a post-PCR denaturation-hybridisation reaction. The single-stranded overhangs are designed to allow directional cloning in a ligase-free manner. This 'enzyme-free cloning' procedure is highly efficient, and is not constrained by the need for the presence of suitable restriction enzyme sites within the plasmid vector. The avoidance of post-amplification enzymatic procedures makes the technique rapid and reliable, avoiding the need for multiple sub-cloning steps.
AB . . . the cloning of PCR products without the need for post-amplification enzymatic treatment. Tailed PCR primer sets are used to create **complementary** staggered overhangs on both **insert** and **vector** by a post-PCR denaturation-hybridisation reaction. The single-stranded overhangs are designed to allow directional cloning in a ligase-free manner. This 'enzyme-free. . .

L2 ANSWER 4 OF 29 MEDLINE on STN DUPLICATE 1
AN 97465967 MEDLINE
DN 97465967 PubMed ID: 9321675
TI Ligation independent cloning irrespective of restriction site compatibility.
AU Li C; Evans R M
CS Gene Expression Laboratory, The Salk Institute for Biological Studies, Howard Hughes Medical Institute, 10010 North Torrey Pines Road, La Jolla, CA 92186, USA.. cli@axpl.salk.edu
NC GM 26444 (NIGMS)

HD 2718 (NICHD)
SO NUCLEIC ACIDS RESEARCH, (1997 Oct 15) 25 (20) 4165-6.
Journal code: 0411011. ISSN: 0305-1048.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199712
ED Entered STN: 19980109
Last Updated on STN: 19980109
Entered Medline: 19971202
AB Here we report the use of exonuclease to expose **complementary** DNA between an **insert** and **vector** such that annealing becomes independent of restriction site compatibility. We demonstrate that unusual and, in some cases, previously impossible cloning strategies can be readily and efficiently achieved as long as the flanking sequences of the linear vectors are highly related. Furthermore, we show that the bacterial repair system resolves the residual mismatches, overhangs or gaps in a predictable fashion to generate excisable inserts. This approach facilitates cloning regardless of restriction site compatibility and overcomes an important limitation in current cloning techniques.
AB Here we report the use of exonuclease to expose **complementary** DNA between an **insert** and **vector** such that annealing becomes independent of restriction site compatibility. We demonstrate that unusual and, in some cases, previously impossible cloning. . .

L2 ANSWER 5 OF 29 CAPLUS COPYRIGHT 2003 ACS on STN
AN 1997:37491 CAPLUS
DN 126:70818
TI The use of PCR for differential screening of cDNA libraries
AU Thomas, Mark G.; Hesse, Sarah A.; Foss, Yvonne J.; Farzaneh, Farzin
CS Department of Biological Anthropology, University of Cambridge, UK
SO Methods in Molecular Biology (Totowa, New Jersey) (1997), 67(PCR Cloning Protocols), 405-418
CODEN: MMBIED; ISSN: 1064-3745
PB Humana
DT Journal
LA English
AB A simple and effective PCR-basic procedure is described for second-round differential screening of phage cDNA libraries. This method allows a large no. of phage isolates to be screened for the presence of differentially expressed sequences even if those isolates are contaminated with phage from neighboring plaques. The procedure involves PCR-amplification of cDNA **inserts** using primers **complementary** to **vector** sequences flanking the cloning site, followed by electrophoresis on duplicate agarose gels and Southern blotting. The duplicate Southern blots can then be probed with cDNA synthesized from mRNA populations isolated under different conditions and the autoradiographs compared for differential expression of the amplified sequences. Each cDNA clone present in a mixed phage isolate will give rise to an independent PCR product of discrete size that will be fractionated and resolved by the gel electrophoresis step. In addn., because mixed phage isolates are tolerated in this method, the initial screening can be carried out using higher than normal plaque densities. Following the screening and identification of the putative clones, it is desirable to obtain sequence information about the differentially expressed cDNAs. The amplification of insert cDNA sequences using a biotinylated version of one of the flanking primers allows the subsequent isolation of single-strand templates using streptavidin-coated magnetic beads and easier sequencing.
AB A simple and effective PCR-basic procedure is described for second-round differential screening of phage cDNA libraries. This method allows a large no. of phage isolates to be screened for the presence of differentially expressed sequences even if those isolates are contaminated

with phage from neighboring plaques. The procedure involves PCR-amplification of cDNA **inserts** using primers **complementary** to **vector** sequences flanking the cloning site, followed by electrophoresis on duplicate agarose gels and Southern blotting. The duplicate Southern blots can then be probed with cDNA synthesized from mRNA populations isolated under different conditions and the autoradiographs compared for differential expression of the amplified sequences. Each cDNA clone present in a mixed phage isolate will give rise to an independent PCR product of discrete size that will be fractionated and resolved by the gel electrophoresis step. In addn., because mixed phage isolates are tolerated in this method, the initial screening can be carried out using higher than normal plaque densities. Following the screening and identification of the putative clones, it is desirable to obtain sequence information about the differentially expressed cDNAs. The amplification of insert cDNA sequences using a biotinylated version of one of the flanking primers allows the subsequent isolation of single-strand templates using streptavidin-coated magnetic beads and easier sequencing.

L2 ANSWER 6 OF 29 MEDLINE on STN DUPLICATE 2
AN 96179060 MEDLINE
DN 96179060 PubMed ID: 8607188
TI Molecular conjugate-mediated gene transfer into isolated human kidneys.
AU Zeigler S T; Kerby J D; Curiel D T; Diethelm A G; Thompson J A
CS Department of Surgery and Gene Therapy Program, School of Medicine, University of Alabama at Birmingham, Birmingham, Alabama 35294 USA.
NC HL09270 (NHLBI)
HL45990 (NHLBI)
HL48491 (NHLBI)
SO TRANSPLANTATION, (1996 Mar 15) 61 (5) 812-7.
Journal code: 0132144. ISSN: 0041-1337.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199605
ED Entered STN: 19960531
Last Updated on STN: 19960531
Entered Medline: 19960523
AB Advances in systemic immunosuppressive therapy for solid organ transplantation have done little to decrease the percentage of allografts that eventually will develop chronic rejection. However, one of the promises of modern molecular biology includes the ability to introduce new genetic information into mammalian hosts. The ability to deliver genes and control their expression in the adult kidney has been described in appropriate animal models. Consequently, gene transfer technology represents a realistic therapeutic approach to modify the allogeneic kidney before engraftment in an effort to decrease the incidence of posttransplant dysfunction. To bridge the gap between animal studies and the clinical application of this technology, we report the first genetic transfection of isolated human kidneys under conditions of organ preservation. Polymerase chain reaction, reversed transcription polymerase chain reaction, and in situ hybridization techniques demonstrated that an adenovirus-polylysine-deoxyribonucleic acid (DNA) complex can be used to **insert** a **complementary** DNA expression **vector** encoding beta-galactosidase into the intact human kidney. Immunohistochemical and in situ enzymatic analyses determined further that gene delivery and expression were localized in proximal tubular epithelial cells. Consequently, targeting of genes to perturb mediators of the local inflammatory response may represent a rational therapeutic interventional strategy in chronic rejection of the kidney.
AB . . . transcription polymerase chain reaction, and in situ hybridization techniques demonstrated that an adenovirus-polylysine-

deoxyribonucleic acid (DNA) complex can be used to **insert** a **complementary** DNA expression **vector** encoding beta-galactosidase into the intact human kidney. Immunohistochemical and in situ enzymatic analyses determined further that gene delivery and expression. . .

L2 ANSWER 7 OF 29 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 1996:215036 BIOSIS
DN PREV199698771165
TI A "double adaptor" method for improved shotgun library construction.
AU Andersson, Bjorn (1); Wentland, Meredith A.; Ricafrente, Jennifer Y.; Liu, Wen; Gibbs, Richard A.
CS (1) Dep. Med. Genet., Biomed. Cent., Box 569, S-751 23 Uppsala Sweden
SO Analytical Biochemistry, (1996) Vol. 236, No. 1, pp. 107-113.
ISSN: 0003-2697.
DT Article
LA English
AB The efficiency of shotgun DNA sequencing depends to a great extent on the quality of the random-subclone libraries used. We here describe a novel "double adaptor" strategy for efficient construction of high-quality shotgun libraries. In this method, randomly sheared and end-repaired fragments are ligated to oligonucleotide adaptors creating 12-base overhangs. Nonphosphorylated oligonucleotides are used, which prevents formation of adaptor dimers and ensures efficient ligation of insert to adaptor. The vector is prepared from a modified M13 vector, by KpnI/PstI digestion followed by ligation to oligonucleotides with ends complementary to the overhangs created in the digest. These adaptors create 5'-overhangs **complementary** to those on the **inserts**. Following annealing of **insert** to **vector**, the DNA is directly used for transformation without a ligation step. This protocol is robust and shows three- to fivefold higher yield of clones compared to previous protocols. No chimeric clones can be detected and the background of clones without an insert is lt 1%. The procedure is rapid and shows potential for automation.
AB. . . digestion followed by ligation to oligonucleotides with ends complementary to the overhangs created in the digest. These adaptors create 5'-overhangs **complementary** to those on the **inserts**. Following annealing of **insert** to **vector** , the DNA is directly used for transformation without a ligation step. This protocol is robust and shows three- to fivefold. . .

L2 ANSWER 8 OF 29 MEDLINE on STN DUPLICATE 3
AN 96000006 MEDLINE
DN 96000006 PubMed ID: 7580902
TI Minimal length requirement of the single-stranded tails for ligation-independent cloning (LIC) of PCR products.
AU Aslanidis C; de Jong P J; Schmitz G
CS Institute for Clinical Chemistry, University of Regensburg, Germany.
SO PCR METHODS AND APPLICATIONS, (1994 Dec) 4 (3) 172-7.
Journal code: 9201445. ISSN: 1054-9803.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199511
ED Entered STN: 19960124
Last Updated on STN: 19980206
Entered Medline: 19951130
AB The ligation-independent cloning of PCR products (LIC-PCR) is a versatile and highly efficient cloning procedure resulting in recombinant clones only. Recombinants are generated between PCR products and a PCR-amplified vector through defined complementary single-stranded (ss) ends artificially generated with T4 DNA polymerase. This procedure does not require restriction enzymes, alkaline phosphatase, or DNA ligase. The

primers used for amplification contain an additional 12-nucleotide sequence at their 5' ends that is **complementary** in the **vector**- and **insert**-specific primers. The (3'-->5') exonuclease activity of T4 DNA polymerase is used in combination with a predetermined dNTP (dGTP for the inserts and dCTP for the vector) to specifically remove 12 nucleotides from each 3' end of the PCR fragments. Because of the complementarity of the ends that are generated, circularization can occur between vector and insert. The recombinant molecules do not require in vitro ligation for efficient bacterial transformation. To make this technique widely applicable, we have simplified the handling of the PCR fragments prior to LIC. The PCR products do not need further purification following the T4 DNA polymerase treatment. Incubation of vector and insert PCR fragments for as little as 5 min is sufficient for a high yield of recombinants. Comparison of the transformation efficiencies using different-length LIC tails revealed that using 12-nucleotide cohesive ends produced four times more transformants than were obtained with the LIC with 10-nucleotide cohesive ends. When the LIC tails were 8 nucleotides long, no transformants were obtained. PCR fragment purification, T4 DNA polymerase treatment, and LIC is complete in < 1 hr.

AB . . . phosphatase, or DNA ligase. The primers used for amplification contain an additional 12-nucleotide sequence at their 5' ends that is **complementary** in the **vector**- and **insert** -specific primers. The (3'-->5') exonuclease activity of T4 DNA polymerase is used in combination with a predetermined dNTP (dGTP for the. . .

L2 ANSWER 9 OF 29 CAPLUS COPYRIGHT 2003 ACS on STN
AN 1995:47001 CAPLUS
DN 122:97804
TI Direct cloning of .lambda.gt11 cDNA inserts into a plasmid vector
AU Poulin, Matthew L.; Chiu, Ing Ming
CS Davis Med. Res. Cent., Ohio State Univ., Columbus, OH, USA
SO Methods in Molecular Biology (Totowa, NJ, United States) (1994), 31(Protocols for Gene Analysis), 9-17
CODEN: MMBIED; ISSN: 1064-3745
DT Journal
LA English
AB A direct method of cloning inserts from .lambda.gt phage into a pBR322 cloning vector is described.
IT Deoxyribonucleic acids
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(**complementary**, direct cloning of .lambda.gt11 cDNA **inserts** into a plasmid **vector**)

L2 ANSWER 10 OF 29 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 1994:416640 BIOSIS
DN PREV199497429640
TI Cloning of **complementary** DNA **inserts** from phage DNA directly into plasmid **vector**.
AU Chiu, Ing-Ming (1); Lehtoma, Kirsten; Poulin, Matthew L.
CS (1) Dep. Intern. Med., Comprehensive Cancer Cent., The Ohio State Univ., Columbus, OH 43210 USA
SO Wu, R. [Editor]. Methods in Enzymology, (1992) Vol. 216, pp. 508-516. Methods in Enzymology; Recombinant DNA, Part G.
Publisher: Academic Press, Inc. 1250 Sixth Ave., San Diego, California 92101, USA.
ISSN: 0076-6879. ISBN: 0-12-182117-X.
DT Book
LA English
TI Cloning of **complementary** DNA **inserts** from phage DNA directly into plasmid **vector**.

L2 ANSWER 11 OF 29 MEDLINE on STN DUPLICATE 4

AN 93125168 MEDLINE
DN 93125168 PubMed ID: 1336101
TI Cloning of **complementary** DNA **inserts** from phage DNA directly into plasmid **vector**.
AU Chiu I M; Lehtoma K; Poulin M L
CS Department of Internal Medicine, Ohio State University, Columbus 43210.
NC K04 CA01369 (NCI)
P30 CA 16058 (NCI)
R01 CA45611 (NCI)
SO METHODS IN ENZYMOLOGY, (1992) 216 508-16.
Journal code: 0212271. ISSN: 0076-6879.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199302
ED Entered STN: 19930226
Last Updated on STN: 19930226
Entered Medline: 19930209
TI Cloning of **complementary** DNA **inserts** from phage DNA directly into plasmid **vector**.

L2 ANSWER 12 OF 29 MEDLINE on STN DUPLICATE 5
AN 92201700 MEDLINE
DN 92201700 PubMed ID: 1339364
TI Ligation-independent cloning of glutathione S-transferase fusion genes for expression in Escherichia coli.
AU Haun R S; Moss J
CS Laboratory of Cellular Metabolism, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD 20892.
SO GENE, (1992 Mar 1) 112 (1) 37-43.
Journal code: 7706761. ISSN: 0378-1119.
CY Netherlands
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
OS GENBANK-M76680; GENBANK-M76682; GENBANK-M79307; GENBANK-M79308; GENBANK-M79309; GENBANK-M79310; GENBANK-M97937; GENBANK-X13839; GENBANK-X60732; GENBANK-X60733
EM 199204
ED Entered STN: 19920509
Last Updated on STN: 20000303
Entered Medline: 19920430
AB A plasmid vector has been constructed that allows the ligation-independent cloning of cDNAs in any reading frame and directs their synthesis in Escherichia coli as glutathione S-transferase-linked fusion proteins. The cloning procedure does not require restriction enzyme digestion of the target sequence and does not introduce any additional sequences between the thrombin cleavage site and the foreign protein. Extended single-stranded tails **complementary** between the **vector** and **insert**, generated by the (3'----5') exonuclease activity of T4 DNA polymerase, obviate the need for in vitro ligation prior to bacterial transformation. This cloning procedure is rapid and highly efficient, and has been used successfully to construct a series of fusion proteins to investigate the sequence requirements for efficient thrombin cleavage.
AB . . . sequence and does not introduce any additional sequences between the thrombin cleavage site and the foreign protein. Extended single-stranded tails **complementary** between the **vector** and **insert**, generated by the (3'----5') exonuclease activity of T4 DNA polymerase, obviate the need for in vitro ligation prior to bacterial. . .

L2 ANSWER 13 OF 29 CAPLUS COPYRIGHT 2003 ACS on STN

AN 1991:529087 CAPLUS
DN 115:129087
TI A rapid and versatile method to transfer an insert between single-stranded vectors and reverse its orientation
AU Adey, Nils B.; Hutchison, Clyde A., III
CS Dep. Microbiol. Immunol., Univ. North Carolina, Chapel Hill, NC, 27599, USA
SO Nucleic Acids Research (1991), 19(12), 3461-2
CODEN: NARHAD; ISSN: 0305-1048
DT Journal
LA English
AB A method was developed to transfer an insert from single-stranded M13mp18 (the donor) into single-stranded pUC119 (the recipient); the resultant insert orientation is opposite that found in the donor. The method is performed as follows: the single-stranded recipient vector is linearized at a specific site using an oligonucleotide and restriction enzyme. The resultant termini are annealed to sequences that flank the insert contained in the single-stranded donor vector. The annealed mols. are simply transformed into competent Escherichia coli then incubated on solid media that selects for the recipient. The vectors tested were M13mp18 as the donor and pUC119 as the recipient but other vector combinations should work. The requirements of the method are: (1) both vectors must contain a single strand origin of replication. (2) It must be possible to select the recipient from the donor (pUC119 encodes ampicillin resistance). (3) The donor and recipient **vectors** must share **complementary** sequences (the polylinkers), the **insert** must lie within or between these sequences, the recipient must be cut within the complementary sequences, and the resultant termini must anneal to regions on the donor that flank the insert of interest. The advantages of this method lie in its versatility and speed. Every insert is treated identically, regardless of what restriction sites it contains. Once a stock of linearized recipient vector is prepd., aliquots are used allowing many inserts to be transferred in a few hours; colonies are obtained the next day.
AB A method was developed to transfer an insert from single-stranded M13mp18 (the donor) into single-stranded pUC119 (the recipient); the resultant insert orientation is opposite that found in the donor. The method is performed as follows: the single-stranded recipient vector is linearized at a specific site using an oligonucleotide and restriction enzyme. The resultant termini are annealed to sequences that flank the insert contained in the single-stranded donor vector. The annealed mols. are simply transformed into competent Escherichia coli then incubated on solid media that selects for the recipient. The vectors tested were M13mp18 as the donor and pUC119 as the recipient but other vector combinations should work. The requirements of the method are: (1) both vectors must contain a single strand origin of replication. (2) It must be possible to select the recipient from the donor (pUC119 encodes ampicillin resistance). (3) The donor and recipient **vectors** must share **complementary** sequences (the polylinkers), the **insert** must lie within or between these sequences, the recipient must be cut within the complementary sequences, and the resultant termini must anneal to regions on the donor that flank the insert of interest. The advantages of this method lie in its versatility and speed. Every insert is treated identically, regardless of what restriction sites it contains. Once a stock of linearized recipient vector is prepd., aliquots are used allowing many inserts to be transferred in a few hours; colonies are obtained the next day.

L2 ANSWER 14 OF 29 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 1992:65495 BIOSIS
DN BR42:29395
TI LOCALIZATION AND CHARACTERIZATION OF A NOVEL NUCLEAR PROTEIN ASSOCIATED WITH THE SPINDLE POLE DURING MITOSIS BY CONFOCAL LASER-SCANNING MICROSCOPY.

AU TANG T K; TANG C-J C; TSOU T-C; WU C-W
CS INST. BIOMED. SCI., ACAD. SINICA, TAIWAN 11529.
SO ABSTRACTS OF PAPERS PRESENTED AT THE THIRTY-FIRST ANNUAL MEETING OF THE AMERICAN SOCIETY FOR CELL BIOLOGY, BOSTON, MASSACHUSETTS, USA, DECEMBER 8-12, 1991. J CELL BIOL. (1991) 115 (3 PART 2), 171A.
CODEN: JCLBA3. ISSN: 0021-9525.
DT Conference
FS BR; OLD
LA English
IT Miscellaneous Descriptors
ABSTRACT BOVINE CELLS MONOCLONAL ANTIBODY **COMPLEMENTARY** DNA **INSERTS** EXPRESSION **VECTOR**

L2 ANSWER 15 OF 29 CAPLUS COPYRIGHT 2003 ACS on STN
AN 1991:529159 CAPLUS
DN 115:129159
TI Methods and compositions for the detection of familial hypercholesterolemia using hybridization probes for human LDL receptor gene
IN Brown, Michael S.; Goldstein, Joseph L.; Russell, David W.
PA University of Texas System, USA
SO U.S., 36 pp. Cont.-in-part of U.S. Ser. No. 687,087.
CODEN: USXXAM
DT Patent
LA English
FAN.CNT 2

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	US 4966837	A	19901030	US 1986-925702	19861030
	US 4745060	A	19880517	US 1984-687087	19841228
	EP 205574	A1	19861230	EP 1986-900490	19851216
	R: BE, DE, FR, IT				
	CA 1280378	A1	19910219	CA 1985-498756	19851230
PRAI	US 1984-687087		19841228		

AB Recombinant DNA transfer **vectors** contg. DNA **inserts** which are **complementary** to either the human low-d. lipoprotein (LDL) receptor gene, or its mRNA transcript, are disclosed. Also disclosed are methods which utilize these genetic probes for diagnosing familial hypercholesterolemia (FH) in a suspected individual. Case studies of individuals are disclosed wherein the genetic deletion mutation is detailed with great precision. The cDNA sequence of the human LDL receptor mRNA and the predicted amino acid sequence of the receptor protein are given.
AB Recombinant DNA transfer **vectors** contg. DNA **inserts** which are **complementary** to either the human low-d. lipoprotein (LDL) receptor gene, or its mRNA transcript, are disclosed. Also disclosed are methods which utilize these genetic probes for diagnosing familial hypercholesterolemia (FH) in a suspected individual. Case studies of individuals are disclosed wherein the genetic deletion mutation is detailed with great precision. The cDNA sequence of the human LDL receptor mRNA and the predicted amino acid sequence of the receptor protein are given.

L2 ANSWER 16 OF 29 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 6
AN 1991:226841 BIOSIS
DN BA91:118301
TI AN EFFICIENT AND SIMPLIFIED METHOD FOR PRODUCING SITE-DIRECTED MUTATIONS BY PCR.
AU BOWMAN S; TISCHFIELD J A; STAMBROOK P J
CS DEP. ANATOMY CELL BIOL., UNIV. CINCINNATI COLL. MED., CINCINNATI, OHIO 45267-0521.
SO TECHNIQUE (PHILA), (1990) 2 (5), 254-260.
CODEN: TCHNEV. ISSN: 1043-4658.

FS BA; OLD
LA English
AB This report describes a simple and general method for introducing site-specific mutations into a cloned DNA fragment. This procedure has been used to introduce eight separate nonsense and missense mutations into the laxZ gene. The strategy requires 1 mutant primer per mutation, 2 commercially available primers, and 2 polymerase chain reaction (PCR) amplifications. The DNA fragment, subcloned into a plasmid polylinker, is PCR-amplified using an oligonucleotide primer (primer 1) containing the desired mutation and a second primer (primer 2) **complementary** to **vector** sequences downstream of the DNA **insert**. An aliquot of the amplification products is added to a second PCR reaction containing primer 2 and a third primer (primer 3) **complementary** to **vector** sequences upstream of the DNA **insert**. An aliquot of the amplification products is added to a second PCR reaction containing primer 2 and a third primer (primer 3) **complementary** to **vector** sequences upstream of the DNA **insert**. The DNA of interest is excised from the final amplified product and subcloned into a second vector for sequence analysis and for subsequent recloning into the DNA from which it was originally derived. The same plasmid construct can be used to generate mutations in any of the cloned sequence simply by substituting the mutant primer.
AB. . . plasmid polylinker, is PCR-amplified using an oligonucleotide primer (primer 1) containing the desired mutation and a second primer (primer 2) **complementary** to **vector** sequences downstream of the DNA **insert**. An aliquot of the amplification products is added to a second PCR reaction containing primer 2 and a third primer (primer 3) **complementary** to **vector** sequences upstream of the DNA **insert**. An aliquot of the amplification products is added to a second PCR reaction containing primer 2 and a third primer (primer 3) **complementary** to **vector** sequences upstream of the DNA **insert**. The DNA of interest is excised from the final amplified product and subcloned into a second vector for sequence analysis. . .

L2 ANSWER 17 OF 29 CAPLUS COPYRIGHT 2003 ACS on STN
AN 1992:144910 CAPLUS
DN 116:144910
TI Rapid verification of lambda-cDNA clones using mixed-base oligonucleotide as screening probe and sequencing primer
AU Au, Lo Chun; Huang, Yue Bin; Teh, Go Wai; Huang, Tur Fu; Choo, Kong Bung
CS Dep. Med. Res., Veterans Gen. Hosp., Taipei, Taiwan
SO Proceedings of the National Science Council, Republic of China, Part B: Life Sciences (1990), 14(4), 233-5
CODEN: PNBSEF; ISSN: 0255-6596
DT Journal
LA English
AB A rapid procedure has been developed for the isolation and verification of cDNA clones isolated from a cDNA library based on lambda vectors. Using information derived from the partial amino acid sequence of a protein, synthetic mixed-base oligonucleotides are first employed as a screening probe using the plaque hybridization procedure. The cDNA inserts of the clones obtained are then directly amplified by polymerase chain reaction (PCR) using primers flanking the cloning site of the vector. Besides being used for cloning into a plasmid vector, the amplified DNAs are also subjected to nucleotide sequence anal. using the same mixed-base oligonucleotides as sequencing primers. This approach allows sequencing through the region of the known amino acid sequence for direct verification of the authenticity of the clones obtained. This procedure has successfully been used for cloning and partial characterization of the gene coding for a platelet aggregation inhibitor.
IT Deoxyribonucleic acid sequence determination
(**complementary**, of **inserts** cloned into lambda **vectors**, mixed-base oligonucleotides for rapid)

L2 ANSWER 18 OF 29 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 1990:471249 BIOSIS
DN BA90:110669
TI DIRECT CLONING OF **COMPLEMENTARY** DNA **INSERTS** FROM LAMBDAGT11 PHAGE DNA INTO A PLASMID **VECTOR** BY A NOVEL AND SIMPLE METHOD.
AU CHIU I-M; LEHTOMA K
CS DEP. INTERNAL MED., OHIO STATE UNIV., DAVIS MEDICAL RESEARCH CENT., 480 W. 9TH AVE., ROOM S2052, COLUMBUS, OHIO 43210.
SO GENET ANAL TECH APPL, (1990) 7 (1), 18-23.
CODEN: GATAEV. ISSN: 1050-3862.
FS BA; OLD
LA English
AB Bacteriophage .lambda.gt11 has been used quite extensively for producing cDNA libraries. The cDNA inserts are usually subcloned into a plasmid vector for large scale production and analysis. However, isolating the recombinant DNA of interest from the phage clones can be a tedious task. Since the E. coli strain Y1088 used for .lambda.gt11 phage infection carries a pBR322-derived plasmid endogenously, we reasoned that this endogenous plasmid could be used directly for cloning the cDNA phage insert. In this report, we describe a method in which cDNA inserts from .lambda.gtii phage were cloned directly into the pBR322 plasmid vector, bypassing the time-consuming procedures of preparing plasmid DNA as a subcloning vector. This method is likely to be extended to the cloning of DNA inserts derived from other phage .lambda. vectors when bacteria containing endogenous pBR322 are used as host cells.
TI DIRECT CLONING OF **COMPLEMENTARY** DNA **INSERTS** FROM LAMBDAGT11 PHAGE DNA INTO A PLASMID **VECTOR** BY A NOVEL AND SIMPLE METHOD.

L2 ANSWER 19 OF 29 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 1989:240958 BIOSIS
DN BA87:122023
TI CONSTRUCTION OF DIRECTIONAL **COMPLEMENTARY** DNA LIBRARIES ENRICHED FOR FULL-LENGTH **INSERTS** IN A TRANSCRIPTION-COMPETENT **VECTOR**.
AU FRECH G C; JOHO R H
CS DEP. PHYSIOL. AND MOL. BIOPHYSICS, BAYLOR COLL. MED., ONE BAYLOR PLAZA, HOUSTON, TEX. 77030.
SO GENE ANAL TECH, (1989) 6 (2), 33-38.
CODEN: GANTDN. ISSN: 0735-0651.
FS BA; OLD
LA English
AB We have designed a simple procedure for the construction of directional cDNA libraries enrhiched for full-length inserts in a transcription-competent cloning vector. An oligonucleotide, its 5' end starting with a heteropolymeric sequence encoding the rare restriction sites for NotI and SfiI, followed by 50 dT residues, is used to prime first-strand synthesis on size-selected mRNA. After second-strand synthesis and EcoRI linker addition, the cDNA is double digested with EcoRI and NotI, or with EcoRI and SfiI, to generate DNA fragments with asymmetric ends that can be directionally cloned. The cDNA fragments are enriched for "full length" byu size selection and ligated into a phage lambda vector containing the T3 and T7 RNA polymerase promotors. These cDNA libraries can directly be used for in vitro synthesis of sense or antisense RNA.
TI CONSTRUCTION OF DIRECTIONAL **COMPLEMENTARY** DNA LIBRARIES ENRICHED FOR FULL-LENGTH **INSERTS** IN A TRANSCRIPTION-COMPETENT **VECTOR**.

L2 ANSWER 20 OF 29 MEDLINE on STN DUPLICATE 7
AN 89057925 MEDLINE
DN 89057925 PubMed ID: 2848261
TI Epstein-Barr virus (EBV) infection of murine L cells expressing recombinant human EBV/C3d receptor.

AU Ahearn J M; Hayward S D; Hickey J C; Fearon D T
CS Division of Molecular and Clinical Rheumatology, Johns Hopkins University School of Medicine, Baltimore, MD 21205.
NC 5K12-DK01298 (NIDDK)
AI22833 (NIAID)
CA4225 (NCI)
SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1988 Dec) 85 (23) 9307-11.
Journal code: 7505876. ISSN: 0027-8424.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 198812
ED Entered STN: 19900308
Last Updated on STN: 19980206
Entered Medline: 19881230
AB The normal host range of Epstein-Barr virus (EBV) is limited to primate B lymphocytes and certain epithelial cells that express the C3d/EBV receptor [complement receptor 2 (CR2, CD21)]. In the present study, expansion of the tissue tropism of EBV has been accomplished by stably transfecting the murine fibroblast L cell line with pMT.CR2. neo.1, a eukaryotic expression **vector** promoting the transcription of a **complementary** DNA **insert** encoding human CR2. High CR2-expressing transfected L cells were selected by fluorescence-activated cell sorting. The recombinant CR2 was shown to have the same molecular weight as wild-type CR2 from Raji cells and to mediate the binding by the transfectants of particles bearing the iC3b and C3d fragments of the third component of complement. All CR2-expressing L cells, but not nontransfected controls, also bound EBV, as assessed by indirect immunofluorescence. After a 60-hr culture, approximately 0.5% of the CR2-expressing cells preincubated with EBV demonstrated immunofluorescent staining of EBV nuclear antigen with serum from a patient with nasopharyngeal carcinoma. No fluorescent staining of cells was seen with monoclonal antibodies to the early antigen complex or to gp350/220, indicating that the infection was predominantly latent. Infected cells cultured for up to 4 weeks remained EBV nuclear antigen-positive. The capacity of recombinant human CR2 to confer on murine L cells susceptibility to stable latent infection by EBV indicates that this receptor is a primary determinant of the tissue tropism of EBV and may facilitate studies of cell-specific factors that regulate the viral growth cycle.
AB . . . of EBV has been accomplished by stably transfecting the murine fibroblast L cell line with pMT.CR2. neo.1, a eukaryotic expression **vector** promoting the transcription of a **complementary** DNA **insert** encoding human CR2. High CR2-expressing transfected L cells were selected by fluorescence-activated cell sorting. The recombinant CR2 was shown to. . .

L2 ANSWER 21 OF 29 MEDLINE on STN DUPLICATE 8
AN 87292094 MEDLINE
DN 87292094 PubMed ID: 3497452
TI Clonal gene therapy: transplanted mouse fibroblast clones express human alpha 1-antitrypsin gene in vivo.
AU Garver R I Jr; Chytil A; Courtney M; Crystal R G
SO SCIENCE, (1987 Aug 14) 237 (4816) 762-4.
Journal code: 0404511. ISSN: 0036-8075.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 198709
ED Entered STN: 19900305
Last Updated on STN: 19900305
Entered Medline: 19870918

AB A retroviral **vector** was used to **insert** human alpha 1-antitrypsin (alpha 1AT) **complementary** DNA into the genome of mouse fibroblasts to create a clonal population of mouse fibroblasts secreting human alpha 1AT. After demonstrating that this clone of fibroblasts produced alpha 1AT after more than 100 population doublings in the absence of selection pressure, the clone was transplanted into the peritoneal cavities of nude mice. When the animals were evaluated 4 weeks later, human alpha 1AT was detected in both sera and the epithelial surface of the lungs. The transplanted clone of fibroblasts could be recovered from the peritoneal cavities of those mice and demonstrated to still be producing human alpha 1AT. Thus, even after removal of selective pressure, a single clone of retroviral vector-infected cells that expressed an exogenous gene in vitro, continued to do so in vivo, and when recovered, continued to produce the product of the exogenous gene.
AB A retroviral **vector** was used to **insert** human alpha 1-antitrypsin (alpha 1AT) **complementary** DNA into the genome of mouse fibroblasts to create a clonal population of mouse fibroblasts secreting human alpha 1AT. After. . .

L2 ANSWER 22 OF 29 CAPLUS COPYRIGHT 2003 ACS on STN
AN 1988:107412 CAPLUS
DN 108:107412
TI Homopolymeric tailing
AU Eschenfeldt, William H.; Puskas, Robert S.; Berger, Shelby L.
CS Div. Cancer Biol. Diagn., Natl. Cancer Inst., Bethesda, MD, 20892, USA
SO Methods in Enzymology (1987), 152(Guide Mol. Cloning Tech.), 337-42
CODEN: MENZAU; ISSN: 0076-6879
DT Journal
LA English
AB A procedure for homopolymeric tailing of insert cDNA and vector DNA is described. The method yields **inserts** with tails of defined length and **vectors** with **complementary** tails having a broader size distribution. The technique may also be used for labeling oligomers.
AB A procedure for homopolymeric tailing of insert cDNA and vector DNA is described. The method yields **inserts** with tails of defined length and **vectors** with **complementary** tails having a broader size distribution. The technique may also be used for labeling oligomers.

L2 ANSWER 23 OF 29 CAPLUS COPYRIGHT 2003 ACS on STN
AN 1987:403795 CAPLUS
DN 107:3795
TI Methods and compositions for the detection of familial hypercholesterolemia
IN Brown, Michael S.; Goldstein, Joseph L.; Russell, David W.
PA University of Texas System, USA
SO PCT Int. Appl., 62 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 2

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 8604090	A1	19860717	WO 1985-US2461	19851216
	W: AT, AU, BB, BG, BR, CH, DE, DK, FI, GB, HU, JP, KP, KR, LK, LU, MC, MG, MW, NL, NO, RO, SD, SE, SU				
	RW: AT, BE, CF, CG, CH, CM, DE, FR, GA, GB, IT, LU, ML, MR, NL, SE, SN, TD, TG				
	US 4745060	A	19880517	US 1984-687087	19841228
	AU 8653065	A1	19860729	AU 1986-53065	19851216
	NL 8520436	A	19861103	NL 1985-20436	19851216
	EP 205574	A1	19861230	EP 1986-900490	19851216
	R: BE, DE, FR, IT				

	BR 8507148	A	19870331	BR 1985-7148	19851216
	HU 41838	A2	19870528	HU 1986-558	19851216
	JP 62501327	T2	19870604	JP 1986-500324	19851216
	DE 3590702	T	19870716	DE 1985-3590702	19851216
	CH 671776	A	19890929	CH 1986-3488	19851216
	CA 1280378	A1	19910219	CA 1985-498756	19851230
	SE 8603591	A	19860826	SE 1986-3591	19860826
	FI 8603474	A	19860827	FI 1986-3474	19860827
	NO 8603438	A	19860827	NO 1986-3438	19860827
	GB 2178743	A1	19870218	GB 1986-20715	19860827
	GB 2178743	B2	19890802		
	DK 8604092	A	19860828	DK 1986-4092	19860828
PRAI	US 1984-687087		19841228		
	WO 1985-US2461		19851216		

AB Recombinant DNA transfer **vectors** contg. DNA **inserts** which are **complementary** to either the human low-d. lipoprotein (LDL) receptor gene or its mRNA transcript are used as genetic probes for diagnosis of familial hypercholesterolemia (FH). The probes are used to detect mutant LDL receptor genes responsible for deficient receptor-mediated endocytosis of LDL by cells of individuals with FH. Close homol. between bovine and human LDL receptor genes allowed use of a cloned bovine cDNA for isolation of part of the human gene from a gene library; this part of the human gene was then used to isolate nearly full-length human cDNA clones. A case study is presented in which the structure of a deletion mutation in the LDL receptor gene of an individual with FH is detd. with great precision by use of genetic probes.

AB Recombinant DNA transfer **vectors** contg. DNA **inserts** which are **complementary** to either the human low-d. lipoprotein (LDL) receptor gene or its mRNA transcript are used as genetic probes for diagnosis of familial hypercholesterolemia (FH). The probes are used to detect mutant LDL receptor genes responsible for deficient receptor-mediated endocytosis of LDL by cells of individuals with FH. Close homol. between bovine and human LDL receptor genes allowed use of a cloned bovine cDNA for isolation of part of the human gene from a gene library; this part of the human gene was then used to isolate nearly full-length human cDNA clones. A case study is presented in which the structure of a deletion mutation in the LDL receptor gene of an individual with FH is detd. with great precision by use of genetic probes.

L2 ANSWER 24 OF 29 CAPLUS COPYRIGHT 2003 ACS on STN
AN 1987:403796 CAPLUS
DN 107:3796
TI Matrix of a DNA detection probe and preparation of the matrix and probe
IN Christophe, Daniel
PA Universite Libre de Bruxelles, Belg.
SO Belg., 19 pp.
CODEN: BEXXAL
DT Patent
LA French
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	BE 904402	A1	19860912	BE 1986-216404	19860312
PRAI	BE 1986-216404		19860312		

AB A matrix for a single-stranded DNA sequence detection probe comprises an immobilized single-stranded DNA vector with a DNA segment complementary to the sequence sought inserted just upstream from a complementary start codon and placed downstream from the immobilized matrix. Its prepn. comprises protecting the DNA **insert** in a recombinant circular single-stranded DNA **vector** with a **complementary** strand running from the start codon region through the **insert** to a restriction site on the **vector**, cleaving the recombinant, immobilizing it or a fragment of it contg. the probe sequence to a solid support such as diazotized paper, and removing the protecting strand under

denaturing conditions. The matrix is then used to prep. many radiolabeled copies of the detection probe which are used to analyze genes. Bacteriophage M13 with a genomic DNA insert was prepd. by std. techniques. The insert was protected by a strand prepd. using DNA polymerase I, etc. before the DNA was partially digested and immobilized on diazobenzyloxymethyl confetti paper. The immobilized DNA was denatured to prep. the matrix. Probes were synthesized off the matrix using DNA polymerase I, [.alpha.-32P]dATP, dCTP, dGTP, and TTP. Each formed probe was freed by incubation in 0.1M NaOH.

AB A matrix for a single-stranded DNA sequence detection probe comprises an immobilized single-stranded DNA vector with a DNA segment complementary to the sequence sought inserted just upstream from a complementary start codon and placed downstream from the immobilized matrix. Its prepn. comprises protecting the DNA **insert** in a recombinant circular single-stranded DNA **vector** with a **complementary** strand running from the start codon region through the **insert** to a restriction site on the **vector**, cleaving the recombinant, immobilizing it or a fragment of it contg. the probe sequence to a solid support such as diazotized paper, and removing the protecting strand under denaturing conditions. The matrix is then used to prep. many radiolabeled copies of the detection probe which are used to analyze genes. Bacteriophage M13 with a genomic DNA insert was prepd. by std. techniques. The insert was protected by a strand prepd. using DNA polymerase I, etc. before the DNA was partially digested and immobilized on diazobenzyloxymethyl confetti paper. The immobilized DNA was denatured to prep. the matrix. Probes were synthesized off the matrix using DNA polymerase I, [.alpha.-32P]dATP, dCTP, dGTP, and TTP. Each formed probe was freed by incubation in 0.1M NaOH.

L2 ANSWER 25 OF 29 MEDLINE on STN DUPLICATE 9
AN 87066733 MEDLINE
DN 87066733 PubMed ID: 3024111
TI Efficient construction of cDNA libraries in plasmid expression vectors using an adaptor strategy.
AU Haymerle H; Herz J; Bressan G M; Frank R; Stanley K K
SO NUCLEIC ACIDS RESEARCH, (1986 Nov 11) 14 (21) 8615-24.
Journal code: 0411011. ISSN: 0305-1048.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 198701
ED Entered STN: 19900302
Last Updated on STN: 19900302
Entered Medline: 19870102

AB We describe a method for the construction of large DNA fragment libraries in plasmid **vectors**, in which **complementary**, single-stranded extensions are ligated onto both **vector** and **insert** DNA using un-phosphorylated adaptor oligonucleotides. Special consideration has been taken of the requirements of expression screening as follows: cDNA synthesis using random oligonucleotide primers is described which maximises the probability of obtaining open reading frame fragments from large mRNA molecules, the adaptors use codons found in high abundance E. coli proteins to minimise problems of premature termination when using strong promoters, and the sequence encoded by the adaptors, when cloned into the bacterial expression vector pEX1, promotes a surface location for the foreign antigenic determinant where it is accessible to antibodies used for screening.

AB We describe a method for the construction of large DNA fragment libraries in plasmid **vectors**, in which **complementary**, single-stranded extensions are ligated onto both **vector** and **insert** DNA using un-phosphorylated adaptor oligonucleotides. Special consideration has been taken of the requirements of expression screening as follows: cDNA synthesis. . .

L2 ANSWER 26 OF 29 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 1985:406795 BIOSIS
DN BA80:76787
TI VECTORS FOR THE DIRECT SELECTION OF COMPLEMENTARY DNA CLONES CORRESPONDING TO MAMMALIAN CELL MESSENGER RNA OF LOW ABUNDANCE.
AU KOWALSKI J; SMITH J H; NG N; DENHARDT D T
CS CANCER RESEARCH LAB., UNIV. WESTERN ONTARIO, LONDON, ONTARIO, CANADA N6A 5B7.
SO GENE (AMST), (1985) 35 (1-2), 45-54.
CODEN: GENED6. ISSN: 0378-1119.
FS BA; OLD
LA English
AB Two c[complementary]DNA cloning vectors which carry the intergenic region of phage f1 and permit efficient cloning (by the Okayama-Berg procedure) of full-length copies of mammalian mRNA in either orientation were constricted. Infection of cells harboring these vectors with f1 phage results in the encapsidation of single-stranded (ss) plasmid DNA carrying the sense or the anti-sense sequence of the cDNA inserts. The **complementary** nature of the cDNA **inserts** in 2 such cDNA libraries facilitates preparative hybridization procedures. These **vectors** have general applicability to any eukaryotic system where changes in the abundance of mRNA transcripts are to be measured and the corresponding cDNA clones isolated.
AB. . . in the encapsidation of single-stranded (ss) plasmid DNA carrying the sense or the anti-sense sequence of the cDNA inserts. The **complementary** nature of the cDNA **inserts** in 2 such cDNA libraries facilitates preparative hybridization procedures. These **vectors** have general applicability to any eukaryotic system where changes in the abundance of mRNA transcripts are to be measured and. . .

L2 ANSWER 27 OF 29 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 1983:283688 BIOSIS
DN BA76:41180
TI A **COMPLEMENTARY** DNA CLONING **VECTOR** THAT PERMITS EXPRESSION OF **COMPLEMENTARY** DNA **INSERTS** IN MAMMALIAN CELLS.
AU OKAYAMA H; BERG P
CS DEPARTMENT OF BIOCHEMISTRY, STANFORD UNIVERSITY MEDICAL CENTER, STANFORD, CALIFORNIA 94305.
SO MOL CELL BIOL, (1983) 3 (2), 280-289.
CODEN: MCEBD4. ISSN: 0270-7306.
FS BA; OLD
LA English
AB A plasmid vector for cloning c[complementary]DNA in Escherichia coli; the same vector also promotes expression of the cDNA segment in mammalian cells. SV40 derived DNA segments are arrayed in the pcD vector to permit transcription, splicing and polyadenylation of the cloned cDNA segment. A DNA fragment containing both the SV40 early region promoter and 2 introns normally used to splice the virus 16S and 19S late mRNA is placed upstream of the cDNA cloning site to ensure transcription and splicing of the cDNA transcripts. An SV40 late region polyadenylation sequence occurs downstream of the cDNA cloning site, so that the cDNA transcript acquires a polyadenylated 3' end. By using pcD-.alpha.-globin cDNA as a model, it was confirmed that the .alpha.-globin transcript produced in transfected cells is initiated correctly, spliced at either of the 2 introns and polyadenylated either at the site coded in the cDNA segment or at the distal SV40 polyadenylation signal. A cDNA clone library constructed with mRNA from SV40-transformed human fibroblasts and this vector (.apprx. 1.4 .times. 106 clones) yielded full-length cDNA clones that express hypoxanthine-guanine phoshoribosyltransferse.
TI A **COMPLEMENTARY** DNA CLONING **VECTOR** THAT PERMITS EXPRESSION OF **COMPLEMENTARY** DNA **INSERTS** IN MAMMALIAN CELLS.

L2 ANSWER 28 OF 29 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 1982:237346 BIOSIS
DN BA74:9826
TI TEMPLATE DIRECTED PAUSING IN IN-VITRO DNA SYNTHESIS BY DNA POLYMERASE ALPHA FROM DROSOPHILA-MELANOGASTER EMBRYOS.
AU KAGUNI L S; CLAYTON D A
CS DEP. PATHOL., STANFORD UNIV. SCH. MED., STANFORD, CALIF. 94305.
SO PROC NATL ACAD SCI U S A, (1982) 79 (4), 983-987.
CODEN: PNASA6. ISSN: 0027-8424.
FS BA; OLD
LA English
AB The activity of D. melanogaster DNA polymerase .alpha. on DNA-primed single-stranded DNA templates was examined. The DNA templates contain a 1471-nucleotide sequence from the heavy-strand origin region of mouse mt[mitochondrial]DNA inserted into the single-stranded bacteriophage vector M13Gori1. Preferred sites for pausing of in vitro DNA synthesis were mapped within the cloned mtDNA **insert** and in the G4 c [**complementary**]DNA strand origin which is contained within the **vector** DNA. Analysis of nascent DNA strands from replicative intermediates has revealed that pause sites are discrete and lie both at the positions of predicted stable dyads and in regions lacking the potential for formation of such structures. The pattern of kinetic pause sites observed for Escherichia coli DNA polymerase III holoenzyme is qualitatively similar to that found for DNA polymerase .alpha.. A subset of the observed kinetic pause signals are recognized by E. coli DNA polymerase I under similar conditions.
AB. . . the single-stranded bacteriophage vector M13Gori1. Preferred sites for pausing of in vitro DNA synthesis were mapped within the cloned mtDNA **insert** and in the G4 c [**complementary**]DNA strand origin which is contained within the **vector** DNA. Analysis of nascent DNA strands from replicative intermediates has revealed that pause sites are discrete and lie both at. . .

L2 ANSWER 29 OF 29 MEDLINE on STN DUPLICATE 10
AN 82211783 MEDLINE
DN 82211783 PubMed ID: 6282692
TI A set of synthetic oligodeoxyribonucleotide primers for DNA sequencing in the plasmid vector pBR322.
AU Wallace R B; Johnson M J; Suggs S V; Miyoshi K; Bhatt R; Itakura K
NC GM07591 (NIGMS)
GM25658 (NIGMS)
GM26391 (NIGMS)
+
SO GENE, (1981 Dec) 16 (1-3) 21-6.
Journal code: 7706761. ISSN: 0378-1119.
CY Netherlands
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 198208
ED Entered STN: 19900317
Last Updated on STN: 19970203
Entered Medline: 19820826
AB Seven oligonucleotide primers complementary to the plasmid vector pBR322 at positions adjacent to five of the unique restriction endonuclease cleavage sites (EcoRI, HindIII, BamHI, SalI and PstI) have been chemically synthesized. The polarity of the primers is such that any DNA inserted at one or a combination of two of the above restriction sites may be sequenced by the chain termination method using one of the synthetic DNA primers. One of the primers for sequencing **inserts** at the PstI site of pBR322 is also **complementary** to the M13 phage **vector** designated bla6. This set of universal primers is useful for rapid sequence determination of DNA cloned into pBR322 or M13bla6.

AB . . . be sequenced by the chain termination method using one of the synthetic DNA primers. One of the primers for sequencing **inserts** at the PstI site of pBR322 is also **complementary** to the M13 phage **vector** designated bla6. This set of universal primers is useful for rapid sequence determination of DNA cloned into pBR322 or M13bla6.

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09/765549

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=> s passmore s?/au
L1 83 PASSMORE S?/AU

=> s l1 and steven
L2 0 L1 AND STEVEN

=> s l1 and recombin#####
L3 8 L1 AND RECOMBIN#####

=> s l3 and homolog###
L4 8 L3 AND HOMOLOG###

=> s l4 and insert#
L5 0 L4 AND INSERT#

=> dup rem l4
PROCESSING COMPLETED FOR L4
L6 4 DUP REM L4 (4 DUPLICATES REMOVED)

=> s l6 and fragment#
L7 4 L6 AND FRAGMENT#

=> d l7 1-4 bib ab kwic

L7 ANSWER 1 OF 4 CAPLUS COPYRIGHT 2003 ACS
AN 1999:704913 CAPLUS
DN 131:332987
TI Multifragment in vivo cloning using **homologous recombination** at sites of partial **homology** for plasmid construction, and use for mutation mapping
IN **Passmore, Steven E.**; Marykwas, Donna L.
PA USA
SO U.S., 27 pp.

CODEN: USXXAM
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	US 5976846	A	19991102	US 1996-584322	19960113
	US 6238923	B1	20010529	US 1999-430911	19991101
	US 6277639	B1	20010821	US 1999-430921	19991101
PRAI	US 1996-584322	A1	19960113		

AB The subject invention relates to a method referred to as multifragment in vivo cloning (MFIVC), wherein the PCR or cleavage by restriction enzyme(s) are used to generate a series of double-stranded DNA **fragments** contg. regions **homologous** to portions of the **fragments** to which they are to be joined, which undergo **recombination** in vivo following transformation into a host with efficient and precise **homologous recombination** (such as the yeast S. cerevisiae). A series is designed so that the last **fragment** in the series contains a region **homologous** to a portion of the first **fragment** in the series, thus forming a circular DNA mol. after **recombination** in vivo. A circular DNA mol. can be selected in vivo if the circular DNA mol. created contains both a suitable DNA replication origin and a suitable marker for genetic selection. A series may be designed so that the first and last **fragment** in the series contain telomeric sequence elements, forming a linear DNA mol. with telomeric sequence elements at its ends, after **recombination** in vivo. One preferred embodiment of this method includes a means for mapping a phenotypically expressed mutation within a gene by detg. which of the multiple **fragments** contain that mutation. A second embodiment of this method includes a means for constructing plasmids using DNA cassettes. A third embodiment of this method includes a means of reasserting mutations in a double-stranded DNA mol. The invention also includes kits contg. reagents for conducting the method.

RE.CNT 12 THERE ARE 12 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

TI Multifragment in vivo cloning using **homologous** **recombination** at sites of partial **homology** for plasmid construction, and use for mutation mapping

IN **Passmore, Steven E.**; Marykwas, Donna L.

AB The subject invention relates to a method referred to as multifragment in vivo cloning (MFIVC), wherein the PCR or cleavage by restriction enzyme(s) are used to generate a series of double-stranded DNA **fragments** contg. regions **homologous** to portions of the **fragments** to which they are to be joined, which undergo **recombination** in vivo following transformation into a host with efficient and precise **homologous recombination** (such as the yeast S. cerevisiae). A series is designed so that the last **fragment** in the series contains a region **homologous** to a portion of the first **fragment** in the series, thus forming a circular DNA mol. after **recombination** in vivo. A circular DNA mol. can be selected in vivo if the circular DNA mol. created contains both a suitable DNA replication origin and a suitable marker for genetic selection. A series may be designed so that the first and last **fragment** in the series contain telomeric sequence elements, forming a linear DNA mol. with telomeric sequence elements at its ends, after **recombination** in vivo. One preferred embodiment of this method includes a means for mapping a phenotypically expressed mutation within a gene by detg. which of the multiple **fragments** contain that mutation. A second embodiment of this method includes a means for constructing plasmids using DNA cassettes. A third embodiment of this method includes a means of reasserting mutations in a double-stranded DNA mol. The invention also includes kits contg. reagents for conducting the method.

ST dsDNA cleavage PCR **recombination** plasmid construction cloning; mutation mapping multifragment vector cloning Saccharomyces

IT DNA
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(cleavage; multifragment in vivo cloning using **homologous recombination** at sites of partial homol. for plasmid construction, and use for mutation mapping)

IT DNA
RL: BPN (Biosynthetic preparation); BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PREP (Preparation); PROC (Process)
(double-stranded, cleavage and **recombination** into plasmids; multifragment in vivo cloning using **homologous recombination** at sites of partial homol. for plasmid construction, and use for mutation mapping)

IT Gene, microbial
RL: ANT (Analyte); BSU (Biological study, unclassified); PRP (Properties); ANST (Analytical study); BIOL (Biological study)
(fliG, mutation mapping; multifragment in vivo cloning using **homologous recombination** at sites of partial homol. for plasmid construction, and use for mutation mapping)

IT Saccharomyces cerevisiae
(**homologous recombination** in; multifragment in vivo cloning using **homologous recombination** at sites of partial homol. for plasmid construction, and use for mutation mapping)

IT **Recombination**, genetic
(**homologous**; multifragment in vivo cloning using **homologous recombination** at sites of partial homol. for plasmid construction, and use for mutation mapping)

IT Genetic markers
(in vectors; multifragment in vivo cloning using **homologous recombination** at sites of partial homol. for plasmid construction, and use for mutation mapping)

IT Plasmids
(linear; multifragment in vivo cloning using **homologous recombination** at sites of partial homol. for plasmid construction, and use for mutation mapping)

IT Mutation
(mapping; multifragment in vivo cloning using **homologous recombination** at sites of partial homol. for plasmid construction, and use for mutation mapping)

IT PCR (polymerase chain reaction)
Plasmid vectors
Transformation, genetic
(multifragment in vivo cloning using **homologous recombination** at sites of partial homol. for plasmid construction, and use for mutation mapping)

IT Primers (nucleic acid)
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(multifragment in vivo cloning using **homologous recombination** at sites of partial homol. for plasmid construction, and use for mutation mapping)

IT Genetic mapping
(of mutations; multifragment in vivo cloning using **homologous recombination** at sites of partial homol. for plasmid construction, and use for mutation mapping)

IT Telomeres (chromosome)
(terminal elements in linear plasmids; multifragment in vivo cloning using **homologous recombination** at sites of partial homol. for plasmid construction, and use for mutation mapping)

IT 249587-75-5, PN: US5976846 SEQID: 1 unclaimed DNA 249587-76-6, PN: US5976846 SEQID: 2 unclaimed DNA 249587-82-4, PN: US5976846 SEQID: 3 unclaimed DNA 249587-83-5, PN: US5976846 SEQID: 4 unclaimed DNA 249587-84-6, PN: US5976846 SEQID: 5 unclaimed DNA 249587-86-8, PN:

US5976846 SEQID: 6 unclaimed DNA 249587-87-9, PN: US5976846 SEQID: 7 unclaimed DNA 249587-88-0, PN: US5976846 SEQID: 8 unclaimed DNA
RL: PRP (Properties)
(unclaimed nucleotide sequence; multifragment in vivo cloning using **homologous recombination** at sites of partial homol. for plasmid construction, and use for mutation mapping)

L7 ANSWER 2 OF 4 CAPLUS COPYRIGHT 2003 ACS
AN 1995:987430 CAPLUS
DN 124:77619
TI Mapping by multifragment cloning in vivo
AU Marykwas, Donna L.; **Passmore Steven E.**
CS Dep. of Molecular and Cellular Biology, Harvard University, Cambridge, MA, 02138, USA
SO Proceedings of the National Academy of Sciences of the United States of America (1995), 92(25), 11701-05
CODEN: PNASA6; ISSN: 0027-8424
PB National Academy of Sciences
DT Journal
LA English
AB An efficient method for mapping mutations is described in which hybrid genes, derived partly from mutant and partly from wild-type DNA, are obtained in vivo by **homologous recombination** of multiple **fragments**. The **recombinants** are formed in a strain in which their phenotypes are immediately apparent. This method was developed to identify changes that disrupt protein-protein interactions demonstrable by the two-hybrid system in yeast. However, it can be extended to any system where **recombination** is possible, provided an assay is available to distinguish between mutant and wild-type phenotypes.
AU Marykwas, Donna L.; **Passmore Steven E.**
AB An efficient method for mapping mutations is described in which hybrid genes, derived partly from mutant and partly from wild-type DNA, are obtained in vivo by **homologous recombination** of multiple **fragments**. The **recombinants** are formed in a strain in which their phenotypes are immediately apparent. This method was developed to identify changes that disrupt protein-protein interactions demonstrable by the two-hybrid system in yeast. However, it can be extended to any system where **recombination** is possible, provided an assay is available to distinguish between mutant and wild-type phenotypes.
ST hybrid gene mutation mapping **recombination** Saccharomyces
IT **Recombination**, genetic
(**homologous**, mapping by multifragment cloning in vivo)

L7 ANSWER 3 OF 4 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 2001:525924 BIOSIS
DN PREV200100525924
TI Method for multifragment in vivo cloning and mutation mapping.
AU **Passmore, Steven E.**; Marykwas, Donna L.
PI US 6238923 May 29, 2001
SO Official Gazette of the United States Patent and Trademark Office Patents, (May 29, 2001) Vol. 1246, No. 5, pp. No Pagination. e-file.
ISSN: 0098-1133.
DT Patent
LA English
AB The subject invention relates to a method referred to as multifragment in vivo cloning (MFIVC). In the method, the polymerase chain reaction or the cleavage by restriction enzyme(s) are used to generate a series of double-stranded DNA **fragments**. Each **fragment** contains a region **homologous** to a portion of the **fragment** to which it is to be joined. These **homologous** regions undergo **recombination** in vivo following transformation into a host with efficient and precise **homologous recombination** (such

as the yeast S. cerevisiae). A series is designed so that the last **fragment** in the series contains a region **homologous** to a portion of the first **fragment** in the series, thus forming a circular DNA molecule after **recombination** in vivo. A circular DNA molecule can be selected in vivo if the circular DNA molecule created contains both a suitable DNA replication origin and a suitable marker for genetic selection. A series may be designed so that the first and last **fragment** in the series contain telomeric sequence elements, forming a linear DNA molecule with telomeric sequence elements at its ends, after **recombination** in vivo. One preferred embodiment of this method includes a means for mapping a phenotypically expressed mutation within a gene. A second embodiment of this method includes a means for constructing plasmids using DNA cassettes. A third embodiment of this method includes a means of reasserting mutations in a double-stranded DNA molecule. The invention also includes kits containing reagents for conducting the method.

AU **Passmore, Steven E.**; Marykwas, Donna L.

AB. . . method, the polymerase chain reaction or the cleavage by restriction enzyme(s) are used to generate a series of double-stranded DNA **fragments**. Each **fragment** contains a region **homologous** to a portion of the **fragment** to which it is to be joined. These **homologous** regions undergo **recombination** in vivo following transformation into a host with efficient and precise **homologous recombination** (such as the yeast S. cerevisiae). A series is designed so that the last **fragment** in the series contains a region **homologous** to a portion of the first **fragment** in the series, thus forming a circular DNA molecule after **recombination** in vivo. A circular DNA molecule can be selected in vivo if the circular DNA molecule created contains both a. . . replication origin and a suitable marker for genetic selection. A series may be designed so that the first and last **fragment** in the series contain telomeric sequence elements, forming a linear DNA molecule with telomeric sequence elements at its ends, after **recombination** in vivo. One preferred embodiment of this method includes a means for mapping a phenotypically expressed mutation within a gene.. . .

L7 ANSWER 4 OF 4 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.

AN 2001:473233 BIOSIS

DN PREV200100473233

TI Methods for multifragment sexual cloning and mutation mapping.

AU **Passmore, Steven E. (1)**; Marykwas, Donna L.

CS (1) 702 Concart St., Hattiesburg, MS, 39401 USA

PI US 6277639 August 21, 2001

SO Official Gazette of the United States Patent and Trademark Office Patents, (Aug. 21, 2001) Vol. 1249, No. 3, pp. No Pagination. e-file. ISSN: 0098-1133.

DT Patent

LA English

AB The subject invention relates to a method referred to as multifragment in vivo cloning (MFIVC), or sexual cloning. In the method, the polymerase chain reaction or the cleavage by restriction enzyme(s) are used to generate a series of double-stranded DNA **fragments**. Each **fragment** contains a region **homologous** to a portion of the **fragment** to which it is to be joined. These **homologous** regions undergo **recombination** in vivo following transformation into a host with efficient and precise **homologous recombination** (such as the yeast S. cerevisiae). One preferred embodiment of this method includes simple rapid means for both mapping phenotypically expressed mutation(s) within a gene and for isolating a gene bearing the relevant mutations(s).

AU **Passmore, Steven E. (1)**; Marykwas, Donna L.

AB. . . method, the polymerase chain reaction or the cleavage by restriction enzyme(s) are used to generate a series of double-stranded DNA

fragments. Each **fragment** contains a region **homologous** to a portion of the **fragment** to which it is to be joined. These **homologous** regions undergo **recombination** in vivo following transformation into a host with efficient and precise **homologous recombination** (such as the yeast S. cerevisiae). One preferred embodiment of this method includes simple rapid means for both mapping phenotypically. . .

IT Major Concepts
Molecular Genetics (Biochemistry and Molecular Biophysics); Methods and Techniques

IT Chemicals & Biochemicals
double-stranded DNA **fragments**: generation

IT . . . Equipment
multifragment sexual cloning: molecular genetic method; mutation mapping: molecular genetic method; polymerase chain reaction: DNA amplification, analytical method, in-situ **recombinant** gene expression detection, sequencing techniques

=>

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> s homolog#### recombin#####(10a) (fragment# or insert#)
L8          705 HOMOLOG#### RECOMBIN#####(10A) (FRAGMENT# OR INSERT#)

=> s l8 and (single or one)
L9          260 L8 AND (SINGLE OR ONE)

=> s l8 and (single fragment# or an insert#)
L10         208 L8 AND (SINGLE FRAGMENT# OR AN INSERT#)

=> s homolog#### recombin#####(10a) (single fragment# or single insert# or one
insert)
L11           1 HOMOLOG#### RECOMBIN#####(10A) (SINGLE FRAGMENT# OR SINGLE INSER
                T# OR ONE INSERT)

=> d l11 bib ab kwic

L11  ANSWER 1 OF 1  EMBASE  COPYRIGHT 2003 ELSEVIER SCI. B.V.
AN   92072917  EMBASE
DN   1992072917
TI   Yeast artificial chromosomes (YACs) and the analysis of complex genomes.
AU   Anand R.
CS   Biotechnology Department, ICI Pharmaceuticals, Alderley Park, Macclesfield
     SK10 4TG, United Kingdom
SO   Trends in Biotechnology, (1992) 10/1-2 (35-40).
     ISSN: 0167-9430  CODEN: TRBIDM
CY   United Kingdom
DT   Journal; (Short Survey)
FS   004     Microbiology
LA   English
SL   English
AB   The development of yeast artificial chromosome (YAC) cloning vectors
     capable of carrying several hundred kilobase-pairs of DNA insert has
     greatly facilitated the study of complex genomes, and the cloning of large
     genes as single fragments. In addition, the ability to
     manipulate YAC sequences by homologous recombination
     makes this system extremely useful for the generation of disease models.
AB   . . . hundred kilobase-pairs of DNA insert has greatly facilitated the
     study of complex genomes, and the cloning of large genes as single
     fragments. In addition, the ability to manipulate YAC sequences by
     homologous recombination makes this system extremely
     useful for the generation of disease models.
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